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What is DNA cloning?

DNA cloning is the isolation of a fragment or fragments of DNA from an organism and placing in a VECTOR that replicates independently of chromosomal DNA. The RECOMBINANT DNA is propagated in a host organism; the resulting CLONES are a set of genetically identical organisms which contain the recombinant DNA

Three main purposes for cloning DNA

1) DNA sequencing
2) Protein production
3) Engineering animals/plants/proteins

Cloning and Expression Vectors

Isolated DNA is cloned into VECTORS for long term storage, propagation of the DNA and for production of protein from gene(s) encoded in the DNA

What are cloning vectors?

Cloning vectors are extra-chromosomal 'replicons' of DNA which can be isolated and can replicate independently of the chromosome. Vectors usually contain a selectable marker - a gene that allows selection of cells carrying the vector e.g. by conferring resistance to a toxin. DNA of interest can be cloned into the vector and replicated in host cells, usually one which has been well characterised.

Commonly used vector systems

• Bacterial plasmids
• Bacteriophages
• Cosmids
• Yeast artificial chromosomes (YACs)
• Ti plasmid (plants)
• Eukaryotic viruses such as baculovirus (insect cells), SV40 virus and retroviruses.

Characteristics of a Cloning Vector

♦ Origin of replication (ORI)

This process marks autonomous replication in vector. ORI is a specific sequence of nucleotide in DNA from where replication starts. When foreign

DNA is linked to this sequence then along with vector replication, foreign (desirable) DNA also starts replicating within host cell.

♦ Selectable Marker

Charecteristics of Selectable marker: A gene whose expression allows one to identify cells that have been transforrned or transfected with a vector containing the **marker gene**. A marker gene is used to determine if a piece of DNA has been successfully inserted into the host organism. Gene usually encoding resistance to an antibiotic. A selectable marker will protect the organism from a **selective agent** that would normally kill it or prevent its growth.

ϖ Restriction sites

Allow cleavage of specific sequence by specific Restriction Endonuclease. Restriction sites in E.coli cloning vector pBR322 include HindIII , EcoRI , BamHI , SalI, PvuI, PstI, ClaI etc. Refer NCERT text book diagram of pBR322

A Cloning Vector that Works with Plant Cells

Most commonly used plant cloning vector **"Ti" plasmid**, or tumor-inducing plasmid. Found in cells of the bacterium known as **Agrobacterium tumefaciens**, normally lives in soil. Bacterium has ability to infect plants and cause a crown gall, or tumorous lump, to form at the site of infection. Ti plasmid - called T DNA - separates from the plasmid and incorporates into the host cell genome. This aspect of Ti plasmid function has made it useful as **a plant cloning vector (natural genetic engineer). Plasmids** are the most commonly used vector system. Several types available for cloning of foreign DNA in the host organism Escherichia coli. Many E. coli plasmids allow the expression of proteins encoded by the cloned DNA

Bacteriophage another common vector system used for cloning DNA. These are viruses which 'infect' E. coli. The M13 bacteriophage is a single-stranded DNA virus which replicates in E. coli in a doublestranded form that can be manipulated like a plasmid. It can be used to produce single-stranded DNA copies which are useful for **DNA sequencing**.

Bacteriophage common vector system used to make DNA libraries. It allows the cloning of larger fragments of DNA than can be incorporated into plasmids.

Transformation is the process by which plasmids (or other DNA) can be introduced into a cell. For E. coli transformation with plasmids is quite straightforward. Plasmids can be introduced by electroporation or by incubation in the presence of divalent cations (usually Ca^{2+}) and a brief heat shock (42°C) which induces the E. coli cells to take up the foreign DNA

1. Two antibiotic selection and replica plating
2. Color selection: blue/white selection using the lacz gene

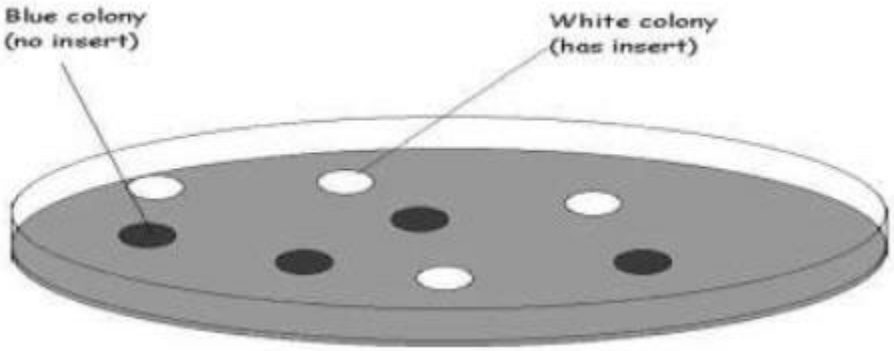
Insertional inactivation :-

Subcloning a DNA fragment into an active gene (usually a marker gene whose function can be easily detected) will disrupt the function of that gene. This can be detected by looking for colonies that no longer display that phenotype.

Colour selection :-

A more common method to determine which transformants contain plasmids with inserts is to use **colour selection**. For E. coli, this involves the **lac complex** and **blue/white screening.**

Colonies carrying plasmid with no insert will be coloured blue whereas colonies carrying recombinant plasmid will be **white**



For plasmids such as pBR322, which contains two antibiotic resistance genes, cloning an insert into one of these will disrupt that gene and inactivate the resistance to that antibiotic.